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CITATION:

SUWA, MASAMI. Nitrogen Mustard N-Oxide Content in the Thoracic Duct Lymph After Arterial Infusion from Celiac Axis. 日本外科宝函 1964, 33(5): 876-895

ISSUE DATE:

1964-09-01

URL:

<http://hdl.handle.net/2433/205754>

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Nitrogen Mustard N-Oxide Content in the Thoracic Duct Lymph After Arterial Infusion from Celiac Axis

by

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Received for Publication July 13, 1964

I INTRODUCTION

Since arterial infusion of anticancer agents was first reported by KLOPP¹⁴⁾¹⁵⁾¹⁶⁾ in 1950, it has been widely used in the field of cancer chemotherapy¹⁾²⁾³⁾⁴⁾⁵⁾⁸⁾¹⁰⁾. Recently SULLIVAN³⁷⁾³⁸⁾³⁹⁾⁴⁰⁾ has improved the method, and showed its clinical usefulness. The therapeutic effects of the treatment are thought to be related to a high concentration of the drug in the tumor tissue. Intraarterial administration of drugs can be performed in various fashions, such as single and repeated injection or infusion, and their combination. The concentration of the drug in tumor tissue must vary according to the method of intraarterial administration. Despite the fact that the drug concentration in the tumor tissue is thought to play an important role in this kind of treatment, few reports have been available regarding the actual drug concentration in the tissue after intraarterial administration of the drugs. Changes in drug concentration in the tissue might be reflected in those in the draining lymph from the tissue. The present study has been attempted to evaluate various methods of intraarterial administration of anticancer agents. The author measured the concentration of nitrogen mustard N-oxide (NMO) in the thoracic duct lymph of male rabbits after the drug was given into the celiac artery by various means of intraarterial administration.

II MATERIALS AND METHODS

A. Method of collecting thoracic duct lymph

1. Animals

Male rabbits (over 2 kg of body weight) were used.

The animals were fed with 500cc of water and 50 g of solid food at 10.00 a. m. every day.

2. Techniques

The animals were injected intravenously with 0.5cc of 5% pentobarbital sodium (Nembutal) per kilogram of body weight and fixed to an operating table in the supine position. A median incision of about 7cm was made over the anterior aspect of the neck, extending to the upper edge of the sternum. After the trachea was exposed, the left internal carotid artery and vagal nerve were isolated as shown in Photos 1 and 2. When this artery was retracted medially, the jugular lymph trunk was ready to be identified, and ligated. After the upper end of the sternum was detached from the muscles, the jugular lymph trunk was followed downward, by use of 10 power magnification of stereoscopic microscope (Model SM 2, Nikon), until the thoracic duct was found at the left edge of

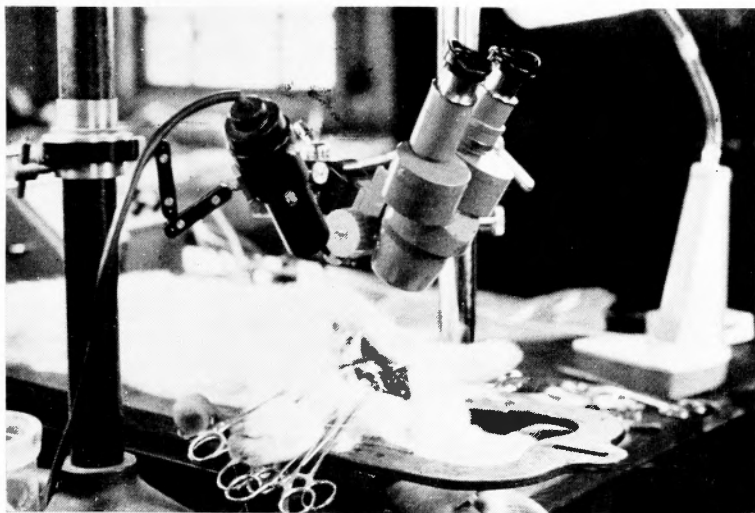


Photo. 1 Stereoscopic microscope ($\times 10$)

the upper end of the sternum (Photo 3, Fig. 1). The stream of lymph was sometimes recognized under the microscope. When it was difficult to find the thoracic duct in the ordinary place, the left clavicle was removed, which made the exploration easier. After two threads (No. 2) were passed under the thoracic duct, a siliconized polyethylene tube (California University Institute No. 3) was inserted into the duct through a small incision made in the anterior wall of the duct between the threads. Lymph from the inserted tube was collected every hour in a separate collecting flask for about 16 hours. During the collection of lymph anesthesia was added by giving 0.5cc of 5% Nembutal, whenever the animal awoke enough to move. In a male rabbit of 2 kilogram body weight, about 2 cc of lymph was obtained every 30 minutes for the first two hours.

B. Method of continuous arterial infusion

1. Techniques of catheterization :

The anatomical relationship of the arteries supplying the stomach of the rabbit is shown in Fig. 2. A No. 3 needle (for intestinal anastomosis) was attached to the tip of a polyethylene tube with an internal diameter of 0.02 in. and external one of 0.03 in. which was smaller than the internal diameter of the celiac artery (Fig. 3).

As shown in Fig. 4, the needle was directly inserted into the aorta 1cm distal to the celiac artery and led to the latter. Then it was taken out through its wall 0.5cm distal to the aorta while the celiac artery was stretched. The top of the polyethylene catheter which followed the needle was cut off and the catheter was drawn back so that its tip might remain within the celiac artery. Bleeding from the wound on the celiac artery was easily controlled by finger tip pressing whereas bleeding from the aortic wall needed some sutures.

2. Apparatus of pressure infusion :

Through the polyethylene tube inserted in the celiac artery, NMO was to be delivered against the arterial pressure. For the purpose of performing pressure infusion, a pressure apparatus (Fig. 5) was used. The infusion pressure which was indicated by the



Photo. 2 Neck structures of rabbit revealed by dissection



Photo. 3 Silicized polyethylene tube inserted into thoracic duct using stereoscopic microscope shown in Photo. 1.

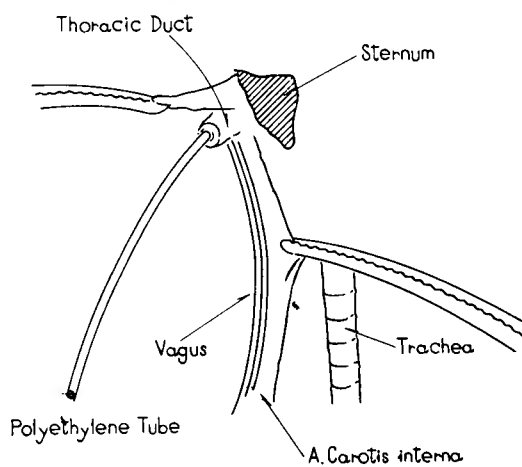


Fig. 1 Explanation of photograph 3

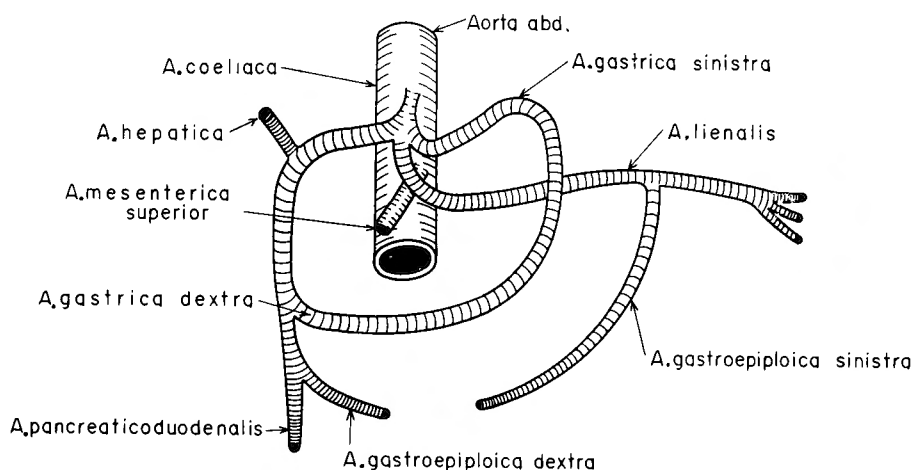


Fig. 2 Arteries arising from celiac axis in rabbit

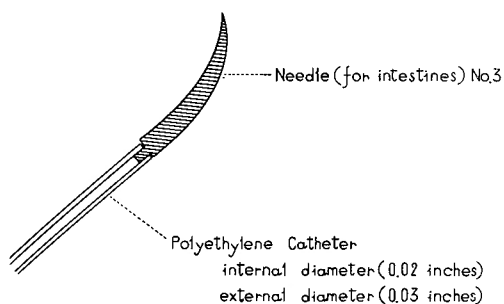


Fig. 3 Tip of catheter

mercury manometer C was kept at about 150mm Hg by the special device A. This was composed of a U-tube which contained mercury, and the upper end of which formed an ampule (Fig. 6). If the mercury column shifted to the left side of the U-tube and air pressure exceeded 150mm Hg in the tube A, air escaped from the ampule, passing through the mercury column as bubbles.

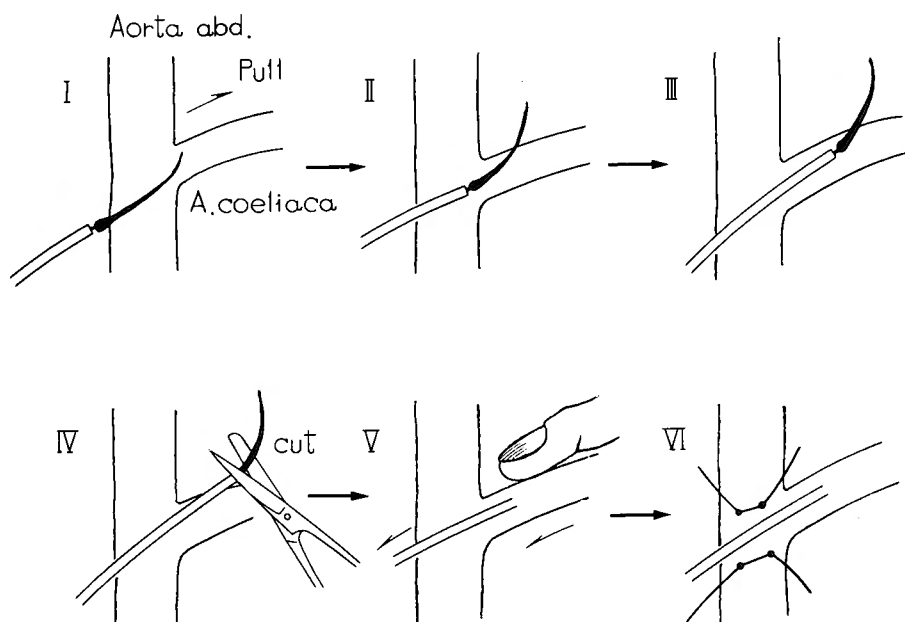


Fig. 4 Insertion of tube into celiac artery

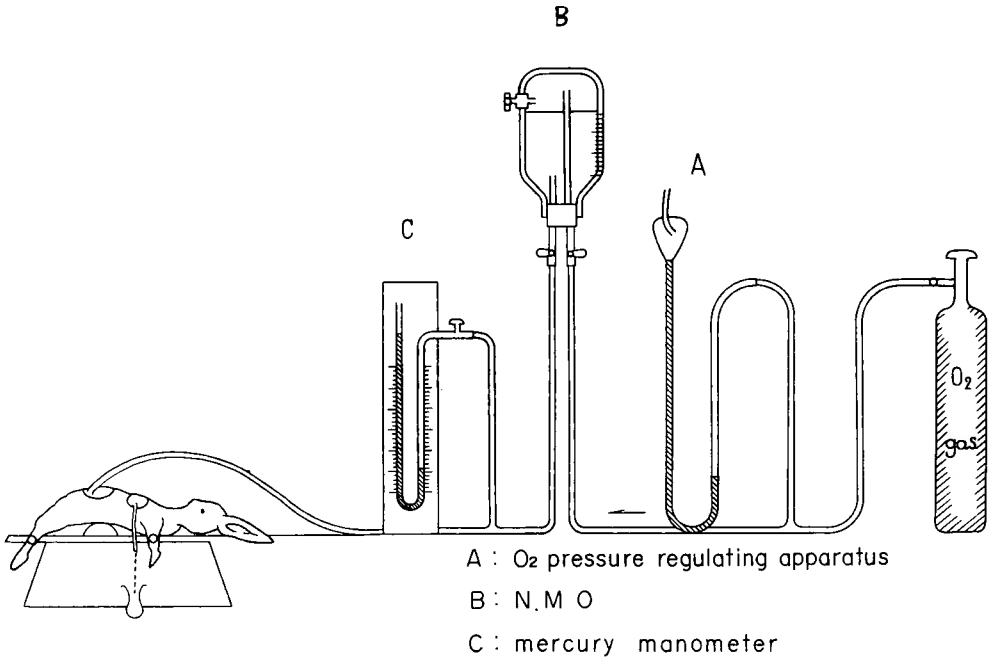


Fig. 5 Apparatus for continuous arterial infusion

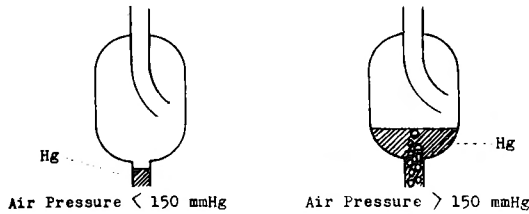


Fig. 6 Detailed illustration of O₂ pressure regulating apparatus

3. Administration of NMO :

A total dose of 10mg of NMO per kilogram of body weight was given each animal excepting one animal in which 40 mg of NMO per kilogram body weight was given as described below in experiment E. The manner of intraarterial administration of the drug was divided into 4 groups : 1) single injection, 2) repeated injections, 3) single infusion, and 4) repeated infusions. If administration of a single dose ends in 5 to 60 seconds, mostly from 5-10 seconds, it is called "injection", whereas such administration as requires more than 1 hour is termed as "infusion". If the total dose is administered at a time, it is referred to as single injection or infusion, while "repeated" is used instead of "single" when the administration is fractionized, the divided dose being given repeatedly. The total dose was diluted in saline at a concentration of 0.5mg/cc. When infused the solution was given at a rate of 40 to 100cc per hour. Dosage schedules for various methods of the drug administration are shown in Tables 1, 3 and 5.

C. Quantitative analysis of NMO

The amount of NMO in the collected lymph was measured by MINAMI's colorimetric

method²³⁾²⁶⁾ by use of bromocresol purple (B. C. P.).

The procedures were as follows :

1. A 5cc sample containing NMO was transferred into a glass stoppered test tube (water added if less than 5cc), then 10cc hexane, 1cc of N-HCl and 1cc of N NaHSO₃ (freshly prepared) were added. The mixture was agitated gently and left alone for 5 minutes.

2. Then 3cc of 15N KOH were added. The mixture was agitated rapidly and centrifuged for 5 minutes at 5000 rpm. The glass stopper was used to prevent evaporation of the solvent.

3. After 5cc of the supernate was transferred to another dried test tube, 0.5cc of B. C. P. reagent was added and mixed well. The mixture transferred to a dried cell, then measured by a spectrophotometer (blank at 410m μ) exactly 1 minute after the addition of B. C. P. reagent.

4. Hexane was used as blank.

5. As reagent blank, distilled water was used instead of the samples and its value was determined in the same way (1-4).

6. The content of NMO was expressed as the value of (4) less the value of (3).

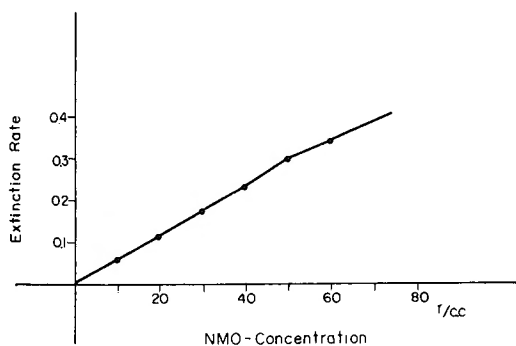


Fig. 7 Extinction-concentration curve for quantitative analysis of NMO

These procedures were applied to a series of samples of which the NMO content was known, and the extinction-concentration curve was made. As shown in Fig. 7, the NMO-concentration in the solution was proportional to its extinction rate according to Beer's law. Therefore the NMO concentration of samples to be tested can be determined from this curve.

III RESULT

The drug dose schedules and results are shown in Tables 1-6 and Figures 8-27. In these figures the left ordinate indicates NMO content in thoracic duct lymph and the right ordinate represents a single dose given to the animals.

A. Single and repeated injections

The drug dose schedules and results for "injection" are shown in Tables (1) and (2).

1) In Case A₁, in which the drug was injected in 10 divided doses at intervals of one hour, the drug concentration in duct lymph was the highest 10 hours after the first injection and it fell to 1 γ /cc 15 hours after the first injection (Fig. 8).

2) In Case A₂, in which the drug was given in 5 divided doses at intervals of 2 hours, the highest concentration of 14 γ /cc was obtained 10 hours after the first injection (Fig. 9).

3) In Case A₃, in which NMO was given in 3 divided dose at an interval of 3

Table 1 NMO dosage schedules for arterial injection

case	* Body Weight	Sex	Single Dose	Time Interval	Total Dose
A ₁	2.1kg	♂	1.0mg/kg	1 hour × 10 *	21mg
A ₂	2.3	♂	2.0	2 × 5	23
A ₃	2.6	♂	3.3	3 × 3	25
A ₄	2.4	♂	5.0	5 × 2	24
A ₅	2.0	♂	10.0	10 × 1	20

* Average of two animals * Injection was repeated ten times at intervals of one hour

Table 2 Hourly changes of NMO content (γ/cc) in thoracic duct lymph after the onset of arterial injection

case	hours	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A ₁		2	3	3	4	5	6	6	6	7	7	6	5	4	2	1		
A ₂		3	4	5	5	7	8	10	10	12	14	11	10	7	3	0		
A ₃		9	10	10	11	14	14	15	16	18	17	15	13	12	9	6	2	0
A ₄		12	14	12	11	10	12	14	7	0								
A ₅		17	18	20	01	1												

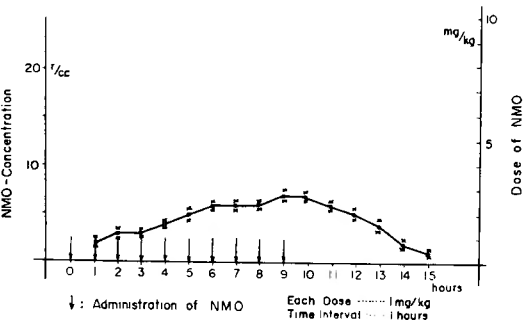


Fig. 8 NMO concentration in thoracic duct lymph in repeated arterial injections (A₁)

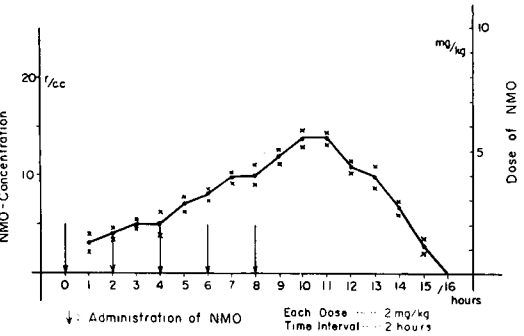


Fig. 9 NMO concentration in thoracic duct lymph in repeated arterial injections (A₂)

hours, the highest concentration of 18γ/cc was seen 9 hours after the first injection and then decreased rapidly. In 17 hours NMO was no longer detected (Fig. 10).

4) In Case A₄, in which NMO was injected in 2 divided doses at an interval of 5 hours, its concentration had two peaks 2 hours after each injection. The value of each peak was 14γ/cc respectively (Fig. 11).

5) In Case A₅, in which the total dose was injected at one time (single injection), the NMO concentration reached its maximum value of 20γ/cc 3 hours after injection and then rapidly fell to 1γ/cc 5 hours after injection (Fig. 12).

B. Single infusion

Tables 3 and 4 show the drug dose schedules and the results for single infusion.

1) In Case B₁, in which the total dose of drug was infused in 10 hours, the highest concentration of NMO (9γ/cc) was obtained 6 hours after the onset of infusion. In 15 hours it fell to 1γ/cc (Fig. 13).

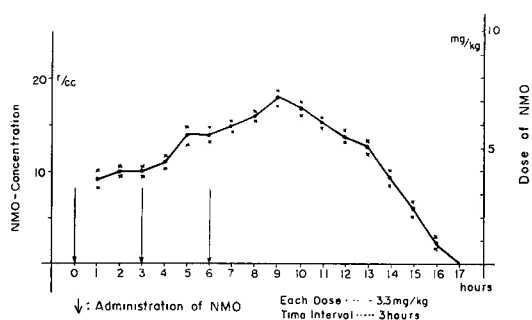


Fig. 10 NMO concentration in thoracic duct lymph in repeated arterial injections (A_3)

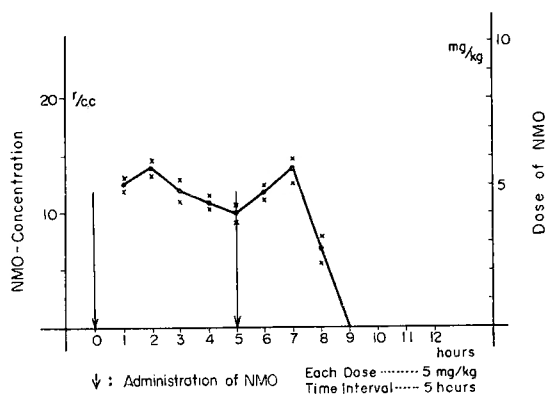


Fig. 11 NMO concentration in thoracic duct lymph in repeated arterial injections (A_1)

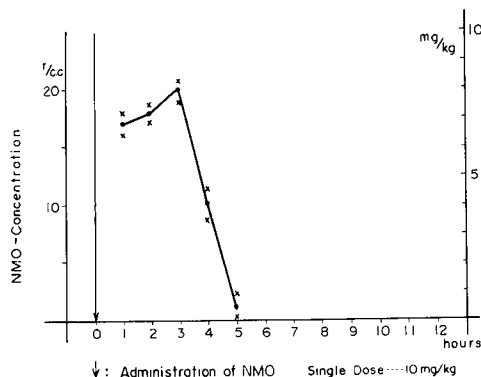


Fig. 12 NMO concentration in thoracic duct lymph in single arterial injection (A_5)

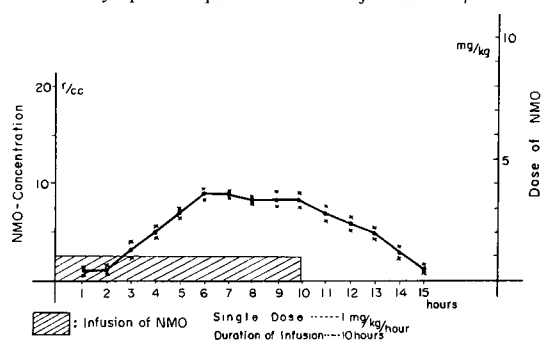


Fig. 13 NMO concentration in thoracic duct lymph in single arterial infusion (B_1)

Table 3 NMO dosage schedules for single arterial infusion

case	Body Weight	Sex	Single Dose	Duration of Infusion	Total Dose
B ₁	2.5kg	♂	1 mg/kg/hour	10 hours	25mg
B ₂	2.2	♂	2	5	22
B ₃	2.1	♂	4	2.5	21
B ₄	2.3	♂	5	2	23
B ₅	2.4	♂	8	1.25	24
B ₆	2.0	♂	10	1	20

Table 4 Hourly changes of NMO content (γ/cc) in thoracic duct lymph after the onset of arterial infusion

case	hour	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B ₁		1	1	3	5	7	9	9	8	8	8	7	6	5	3	1
B ₂		3	5	6	8	8	9	8	7	2						
B ₃		4	7	10	12	12	8	5	3	2	1					
B ₄		4	7	10	6	3	2	1								
B ₅		16	24	26	14	10	6	0								
B ₆		20	21	20	16	11	5	0								

2) In Case B₂, the highest concentration of 9γ/cc was reached 6 hours after the onset of infusion (Fig. 14).

3) In Case B₃, the highest concentration of 12γ/cc was obtained between 4 and 5 hours after the onset of infusion. The concentration of NMO decreased rapidly in 5 to 6 hours (Fig. 15).

4) In Case B₄, the concentration curve showed a peak with a value of 12γ/cc 3 hours after the onset of infusion, and then decreased to 1γ/cc 7 hours after the onset of infusion (Fig. 16).

5) In Case B₅, the highest concentration of 26γ/cc was obtained 3 hours after the onset of infusion (Fig. 17).

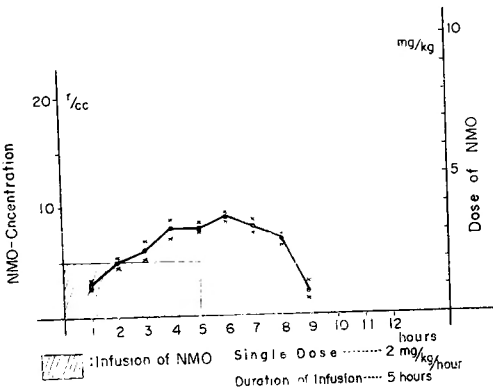


Fig. 14 NMO concentration in thoracic duct lymph in single arterial infusion (B₂)

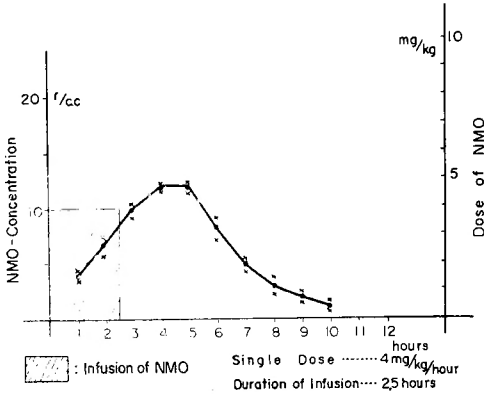


Fig. 15 NMO concentration in thoracic duct lymph in single arterial infusion (B₃)

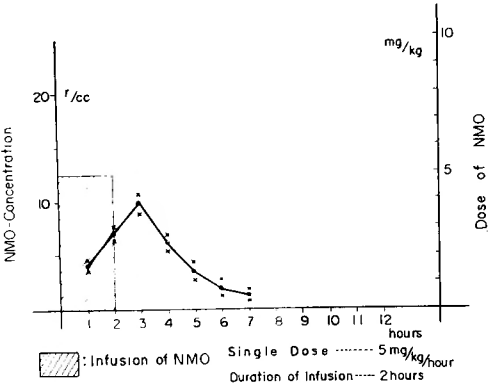


Fig. 16 NMO concentration in thoracic duct lymph in single arterial infusion (B₄)

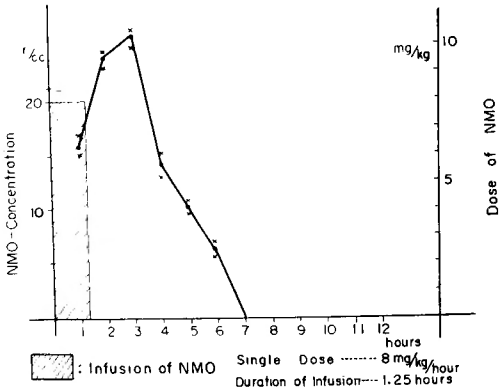


Fig. 17 NMO concentration in thoracic duct lymph in single arterial infusion (B₅)

6) In Case B₆, the highest concentration was 21γ/cc 2 hours after the onset of infusion (Fig. 18).

C. Repeated infusions

The dose schedules and results for repeated infusions are shown in Tables 5 and 6.

1) In Case C₁, a single dose of 2mg/kg was infused for an hour four times with

intervals of one hour. The highest concentration of $5.5\gamma/\text{cc}$ was reached 5 hours after the onset of the first infusion (Fig. 19).

2) In Case C_2 , in which a single dose of 5 mg/kg was infused for an hour twice with a pause of 4 hours, the highest concentration of $11\gamma/\text{cc}$ was obtained 6 hours after onset of the first infusion (Fig. 20).

D. Single and repeated infusion through a polyethylene tube inserted into the aorta retrogradely from the femoral artery

In this experiment a polyethylene tube was inserted into the aorta retrogradely from the femoral artery and its tip was maintained at the level of the celiac axis. Through the tube the drug was administered according to the same dose schedules as

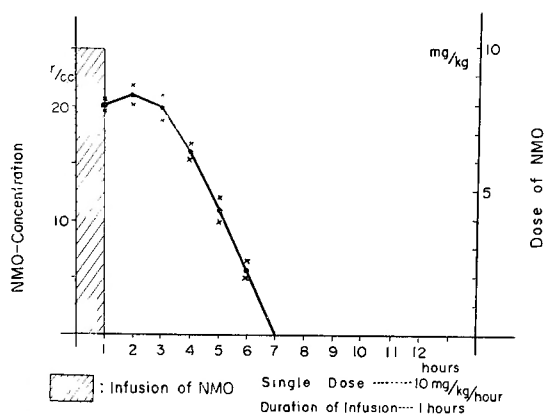


Fig. 18 NMO concentration in thoracic duct lymph in single arterial infusion (B_6)

Table 5 NMO dosage schedules for repeated arterial infusion

case	Body Weight	Sex	Single Dose	Duration of Infusion	Time Interval	Total Dose
C_1	2.5kg	♂	2 mg/kg/hour	1 hour \times 5	1 hour \times 5	25mg
C_2	2.3	♂	5	1 \times 2		23

+ Infusion of the duration of one hours was repeated five times with intervals of one hour.

Table 6 Hourly changes of NMO content (γ/cc) in thoracic duct lymph after the onset of repeated arterial infusion

case	hours	1	2	3	4	5	6	7	8	9	10	11	12	13
C_1		4	3	6	4	5	4	6	4	4	3	2	1	
C_2		9	8	7	5	4	11	9	8	4	3	2	1	

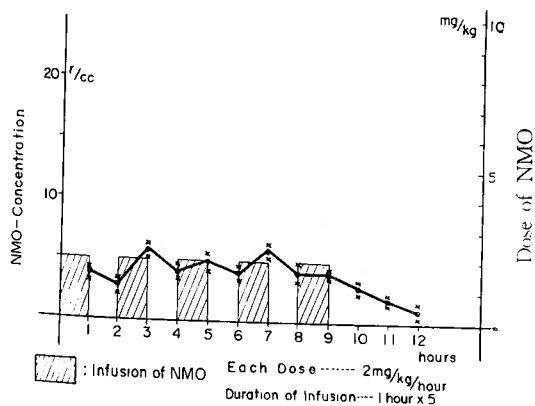


Fig. 19 NMO concentration in thoracic duct lymph in repeated arterial infusion (C_1)

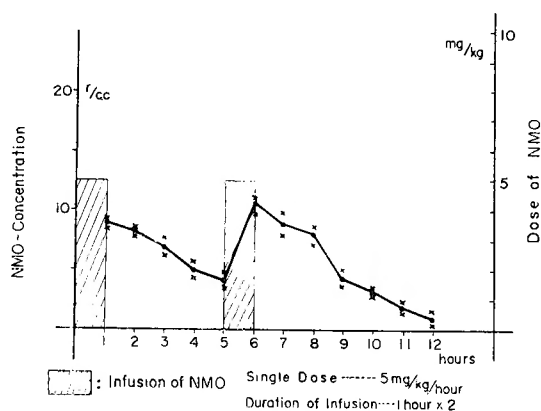


Fig. 20 NMO concentration in thoracic duct lymph in repeated arterial infusion (C_2)

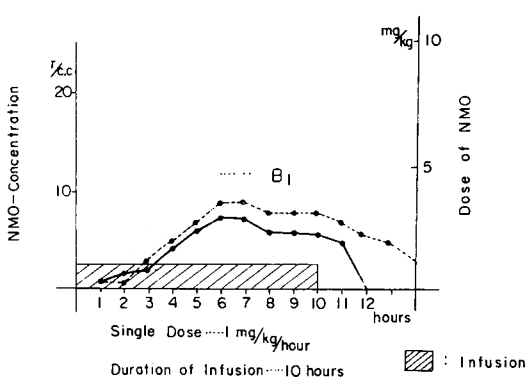


Fig. 2 1

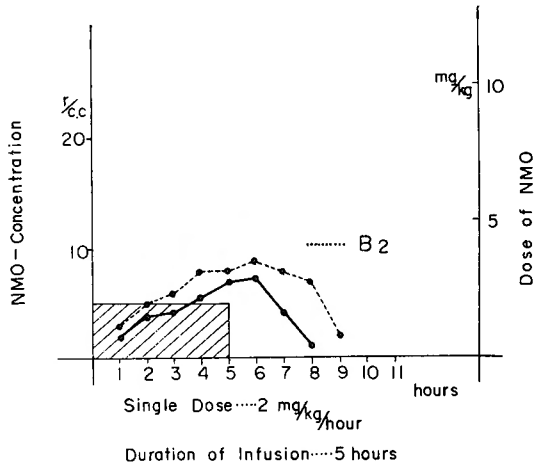


Fig. 2 2

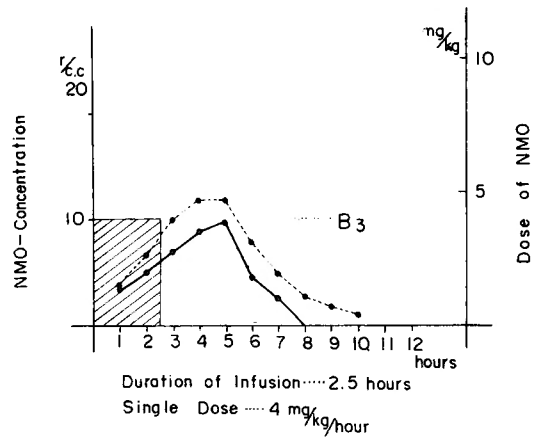


Fig. 2 3

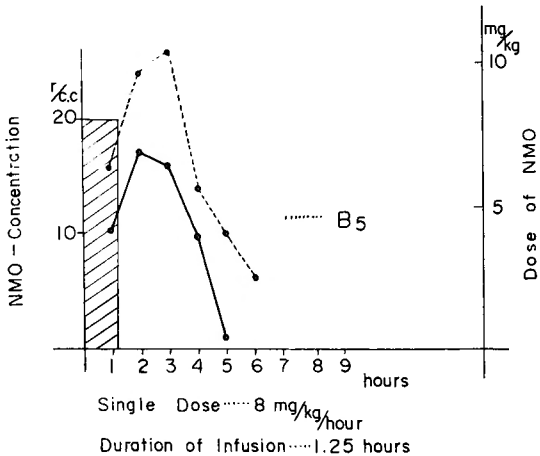


Fig. 2 4

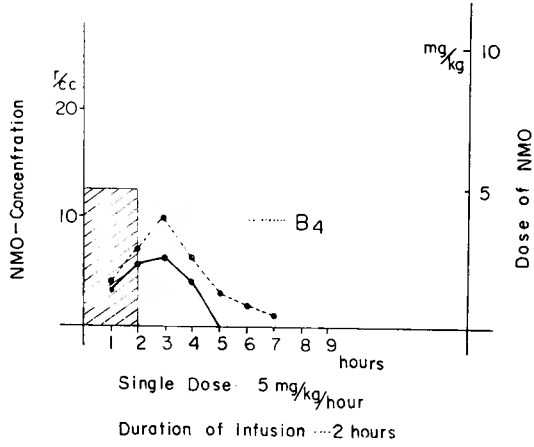


Fig. 2 5

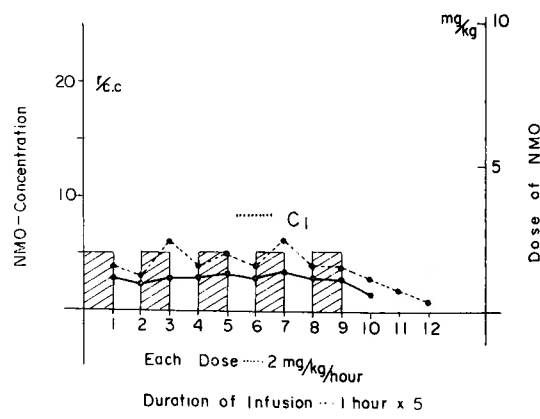


Fig. 2 6

Fig. 21-26 Comparison between results obtained by direct intubation into the celiac artery and retrograde femoral intubation into the aorta
Dotted line : direct intubation
Solid line : retrograde intubation

those in Tables 3 and 5. The results are shown in Figures 21-26, in which the solid lines represent the drug concentration in duct lymph in the cases of retrograde intubation into the aorta and the dotted lines to be compared show the results in the cases of direct intubation into the celiac artery. In all cases, the drug concentration in duct lymph was higher when the tube was inserted directly into the celiac artery.

E. Repeated infusions of a highly concentrated solution of NMO

A total dose of 40 mg of NMO per kilogram body weight was dissolved in 100 cc of saline, which was divided into four doses. The divided dose of 10 mg/kg was infused four times with intervals of one hour. The concentration of the drug in duct lymph showed a peak value of 30 γ /cc 5 hours after the onset of the first infusion and decreased thereafter (Fig. 27). In this case the blood pressure fell to 70 mmHg about 12 hours after the onset of the first infusion.

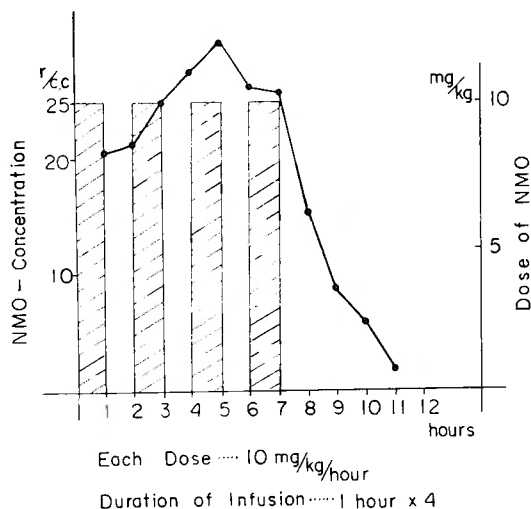


Fig. 27 NMO concentration in thoracic duct lymph in repeated arterial infusion with large amount of NMO

IV DISCUSSION

Chemotherapy of malignant tumors is still far from satisfactory, whether cell poisons, metabolic antagonists or antibiotics are employed. Although it seems impossible at present to cure malignant neoplasms by chemotherapy alone, many investigators have been trying to increase the effect of anticancer agents with minimum side effects. In 1955, KLOPP et al.⁽¹⁴⁾⁽¹⁵⁾⁽¹⁶⁾ reported a new method of administering anticancer agents intraarterially. They gave nitrogen mustard to rats which were bearing Hardrian gland carcinoma No. 2226 or pseudomucinous adenopapillary carcinoma of the ovary, and noticed remarkable degenerative changes of the tumor cells compared with the results obtained by usual intravenous administration of the drug. Since then intraarterial injection of various alkylating agents such as nitrogen mustard and its N-oxide compound (NMO) has been studied by many investigators⁽¹⁾⁽²⁾⁽³⁾⁽⁴⁾⁽⁵⁾⁽⁸⁾⁽⁹⁾⁽¹⁰⁾⁽¹²⁾⁽¹⁹⁾⁽³⁰⁾⁽³¹⁾⁽³⁴⁾⁽³⁵⁾⁽³⁶⁾. Recently SULLIVAN⁽³⁷⁾⁽³⁸⁾⁽³⁹⁾⁽⁴⁰⁾ has reported excellent results obtained by arterial continuous infusion chemotherapy by use of a special pump. All these investigators have been intending to give a higher concentration of the drugs to the tumor tissue. To improve the effect of the drugs given in this way, it is indispensable to know at what concentration and how long the administered chemotherapeutic remains in the tumor tissue before excreted or decomposed. As DRUCKREY⁽⁶⁾⁽⁷⁾ pointed out, the effects of chemotherapy depend on the sites of action and the duration of effective concentration of the chemotherapeutic agent.

There is no need to say that all the drug administered intraarterially does not necessarily enter into the tumor tissue nourished by the artery. Some amount of the drug

which has entered into the tumor tissue is thought to be drained through the lymphatic vessels and the changes in the drug concentration in the draining lymph might be correlated to those in the tumor tissue. Therefore, measurement of the drug concentration in draining lymph might give more valuable, although indirect, information on the changes in drug concentration in the tumor tissue itself.

Although there have been many reports concerning plasma or urinary concentration of anticancer agents administered in usual ways such as intravenous administration, few authors have dealt with their lymph concentration. In regard to arterial infusion, almost no work has been on the concentration of anticancer agents in lymph except one by TOKUOKA⁽¹⁾⁽⁴²⁾ who measured the lymphatic content of nitromin in a dog. He inserted a polyethylene tube in the aorta, through which nitromin was given by single infusion. In the present experiment NMO was given to male rabbits intraarterially in varying fashions, and then the concentration of NMO in thoracic duct lymph was measured.

Consideration of experimental animals : Female rabbits were avoided because humoral changes in pregnancy might affect experimental results. When a large amount of fatty food was given to facilitate visualization of the thoracic duct, duct lymph contained many fat globules, which made the measurement of NMO content in the samples difficult. Therefore, the animals were fed regularly with 50 gram of solid material and 500cc of water at a certain time once in the morning.

Factors affecting thoracic duct lymph flow : When the amount of NMO in the unit volume of thoracic duct lymph is to be examined as in this experiment, every factor which affects normal flow rate of duct lymph should be avoided. Although the factors having influence on the lymph flow rate have not been fully understood, the following are given by REINHARDT⁽²⁹⁾ : i) autonomic nervous system, ii) circulating blood volume, iii) electrolyte balance, and iv) osmotic pressure. When the abdomen is opened and the major splanchnic nerve is involved, changes in the tonus of the autonomic nervous system is thought to result in the changes in the lymph flow rate. Therefore, in this experiment, the abdomen was closed soon after the polyethylene catheter was positioned in place. Because movement of the animals would result in changes in flow rate of the duct lymph, the animals were anaesthetized. A constant level of anesthesia was maintained with 0.5cc of 5% Nembutal injected every 2 hours intravenously.

As to the effect of circulating blood volume, KOTANI and SHIGEMOTO⁽³²⁾ observed a rapid and prolonged increase in lymph flow in rabbits when 180-200cc of saline was infused at a rate of 1cc per minute. A large amount of fluid should not be given, when a constant flow rate of lymph is required. In this experiment, the total

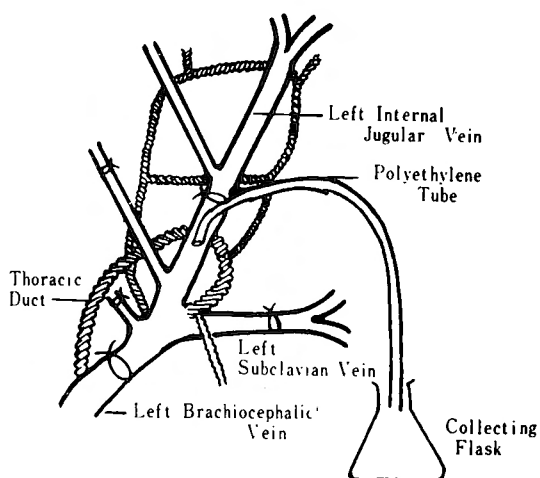


Fig. 28 Indirect method of collecting chyle
(T. Shimizu)⁽³³⁾

amount of NMO solution given to the animals was limited to less than 40cc and 100cc in injection and "infusion" respectively. When 100cc of the NMO solution was infused, the rate of infusion was less than 100cc per hour.

Contamination of lymph with blood : If the indirect method of collecting lymph

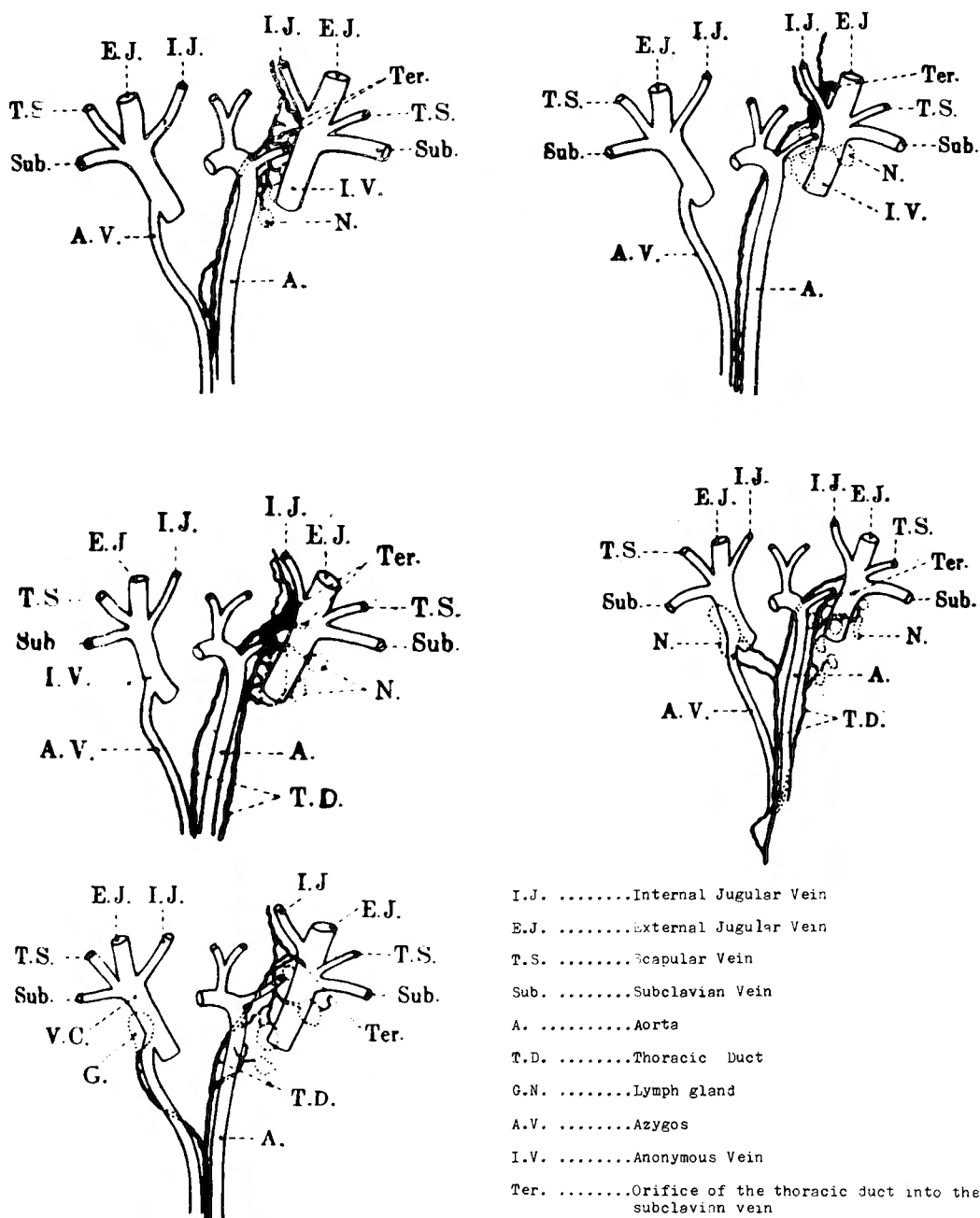


Fig. 29 Anomalies of thoracic duct in rabbits (Kistler, R.H.)¹³⁾

through the left subclavian vein is used (Fig. 28), the sample is often contaminated with blood. In addition, anatomical anomalies of the junction of the thoracic duct with the vein occasionally seen in rabbits (Fig. 29)^{13) 17) 20) 21) 22) 24) 27) 28) 43)} may cause difficulty in collecting lymph by the indirect method³³⁾. Therefore the author isolated the thoracic duct, into which a siliconized polyethylene tube was inserted directly. Pure lymph obtained by the direct collecting method did not need correction for blood contamination in evaluating NMO content in the samples.

Quantitative analysis of NMO : NMO can be quantitatively determined by various methods, for example, radioactive tracer technique^{18) 25)}, chemical analysis, or bioassay. The chemical analysis method which can give the figures of active form of NMO was employed in this experiment.

Because activity of NMO decreases by one half in 4 hours in saline solution (Fig. 30)⁴¹⁾, NMO solution should be freshly prepared every 3 to 4 hours prior to administration.

When the drug was given through the catheter inserted directly into the celiac axis, the concentration of the drug in thoracic duct lymph was obviously higher than when the drug was administered through the catheter introduced into the aorta. It can be said that the drug should be given directly into the feeding artery, when a higher concentration of the drug in the tissue is required.

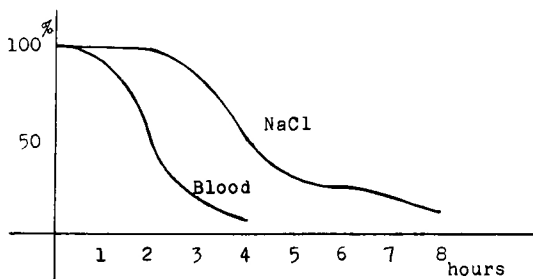


Fig. 30 Rate of decrease of NMO in saline and blood of mouse (Tokuoka, J.)⁴¹⁾

Drug concentration in thoracic duct lymph : In our experiment in which the total doses of the drug was given by "single injection", the drug concentration in the duct lymph an hour after the injection was as high as 17 γ /cc, and 3 hours after injection it reached the highest level at 20 γ /cc. Thereafter it decreased and became less than 1 γ /cc in 5 hours after injection. In contrast, when the drug was given in ten divided doses at intervals of one hour, the drug concentration rose gradually and reached the highest level from 9 to 10 hours after the first injection. After the last injection of the divided dose, the decrease in the drug concentration was not so rapid as seen in the case of "single injection" and the period of 6 hours elapsed before the drug concentration reached the level of 1 γ /cc.

When the drug was given in two divided doses, the curve of the drug concentration showed two peaks, each two hours after injection, and decreased to about zero 4 hours after the second injection. When the drug was given in 3 or 5 divided doses, a considerably high level of the drug concentration was maintained for a long period and the highest level was obtained 3 hours after the last injection in both cases. Thereafter it needed 8 or 11 hours for the drug concentration to reach the zero line. From these results it might be said that when the drug was given in three divided doses, it was retained in the tissue for a while, to be released into the thoracic duct lymph thereafter. In regard to maintenance of a higher level of the drug concentration, the injections with three di-

vided doses at intervals of 3 hours seem to be best. In addition, in these cases the high drug concentration lasted for the longest period.

Suppose the drug concentration in the lymph is in proportion to that in the tissue, the results described above might indicate that a given amount of drug should be administered in divided doses at intervals of about 3 hours when a higher drug concentration is to be given to the tissue for a longer period.

When the drug was given by single infusion for 10 hours, the drug concentration in the duct lymph rose gradually and reached the highest level 6 hours after the beginning of infusion. Then it remained almost at the same level during the period of infusion, to reduce gradually and reach about zero 5 hours after the end of infusion. The plateau of the concentration curve might indicate that the drug in the tissue was keeping a balance between transmission from blood and release into lymph. When the same amount of drug was infused for one hour or so, the curve of the drug concentration was similar to that of single injection. The drug was found in the duct lymph a little longer than in the case of single injection.

When the drug was infused for periods varying from 1.25, 2, 2.5 and 5 hours, the drug concentration curve increased more slowly with a lower value of the highest level. After the end of infusion, the drug concentration reached almost the zero line in 5, 7 and 4 hours respectively. In all cases of these single infusions, the highest level of the drug concentration appeared one or two hours after the end of infusion, excepting in the case of single infusion for 10 hours. A similar response was seen in the cases of repeated injection, which gave the highest drug concentration a little later, that is, 2-3 hours after the last injection. The time necessary for the lymph in the tissue to reach the cervical portion of the thoracic duct must affect to some extent the retardation of the highest drug concentration. Difference in the time of appearance of the highest drug concentration between the infusion and injection cases might also indicate that a larger amount of the drug was retained in the tissue when the drug was given by injection. To recapitulate, single infusion for varying periods with the same total dose did not give any benefit compared with single or repeated injections.

Infusion was also done with the same amount of drug dissolved in the same amount of saline, divided into two or five parts, with each lasting for one hour. When the drug was given by two fractionized infusions the curve of the drug concentration did not show any peak after each infusion in contrast to the case of injection which was divided. It might be suggested that when the concentration differential of drug across the blood vessel wall is small the drug is retained in small amounts in the tissue. When a given amount of drug is given by injection, it might induce a larger concentration differential across the blood vessel wall in the tumor tissue when compared with the case of infusion of the same amount of drug. This does not necessarily mean that the larger the concentration differential, the more the drug is transported into the tissue. When the drug concentration in blood is too large, it will affect permeability of the blood vessel wall, which might result in reducing the rate of transportation of the drug into the tissue. An appropriate single dose in this respect has not yet been examined. Any way, infusion method does not seem to give any benefit when compared with injection method when a higher drug con-

centration in tumor tissue is required for a longer period. On the other hand, when a constant level of drug concentration for a longer period is needed, for example in the case of administration of such an antimetabolite as methotrexate, continuous infusion might be best for maintaining such a plateau as seen in Fig. 13.

V CONCLUSION

After a total dose of 10mg of NMO per kilogram body weight was administered intermittently or continuously through a polyethylene catheter inserted into the celiac axis of male rabbits weighing from 2 to 2.5kg, thoracic duct lymph was collected continuously. The amount of NMO in thoracic duct lymph was colorimetrically determined by the hexane bromocresol purple method. The results were as follows :

1) The concentration of NMO in thoracic duct lymph was higher when the drug was administered through a catheter inserted into the celiac axis than when administered through a catheter introduced retrogradely into the aorta from the femoral artery to the level of the celiac artery.

2) When the total dose was injected at a time or infused in an hour, the NMO concentration reached a maximal value of 20 γ /cc 3 hours after the onset of administration. Although a higher concentration lasted slightly longer in the case of single infusion than in the case of single injection, it decreased rapidly within a short period of time in both cases.

3) When the total dose was injected in ten divided doses at intervals of one hour or infused for the period of ten hours, a constant level of the drug concentration was maintained for a longer period.

4) When the total dose was injected in divided doses at intervals of 3 to 4 hours a considerably higher level of the drug concentration was obtained for the same period of time as when the drug was given for the period of 10 hours.

5) As activity of NMO decreases by one half in 4 hours in saline solution, the solution should be prepared within 3 hours before use. Consequently, repeated intraarterial injection of freshly prepared NMO solution every 3 to 4 hours seems to be the most effective method of administration to maintain a higher drug concentration in lymph for a longer period.

Presented in part before the 22nd General Assembly of the Japanese Cancer Association, Okayama, Japan, October 20 and 21, 1963.

The author wishes to gratefully acknowledge the cordial advice and guidance of Professor CHISATO ARAKI, M. D., Assistant Professor IKUZO YOKOYAMA, M. D. and Instructor RYOICHI TSUCHIYA, M. D. in the First Surgical Division, Kyoto University Medical School.

I am indebted to Professor ISOO HORII, M. D., Assistant Professor M. KOTANI, M. D., K. SHIGEMOTO, M. D. in the Department of Anatomy, Kyoto University for many thoughtfully extended courtesies, J. P. SATTERWHITE, M. D. in the Japan Baptist Hospital in Kyoto for valuable assistance and I wish to thank SHUNZO MAETANI, M. D. in the Second Surgical Division, Kyoto University Medical School for his suggestions.

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和文抄録

腹腔動脈内 Nitrogen Mustard N-Oxide 注入時に 於ける胸管リンパ液内濃度

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諏 訪 正 美

近時、制癌剤の効果を高め、かつ、副作用を少なくする目的で、罹患部位を養う動脈内に、制癌剤の投与を行なう療法が発達して来た。本療法により期待される制癌効果の向上は、対象組織内に移行する制癌剤の濃度が高い事によるものである。動脈内に制癌剤を投与する場合、各種の投与方式が考慮されるが、適切な投与方式を決定するためには、個々の投与方法により、実際に組織内に移行する薬剤濃度の消長を知る必要がある。組織内に移行した薬剤の一部は、リンパ液に移行するから、動脈内に薬剤を投与後、その動脈が養なっている領域から流出するリンパ液内の薬剤濃度の変化を測定することによって、間接的に当該組織内の薬剤濃度の消長を察知することが出来るであろう。

体重2.0～2.5kgの雄性家兎を使用し、腹腔動脈基部内にCatheterを留置し、これを通して制癌剤 Nitrogen Mustard N-Oxideの投与を、間歇的あるいは持続的に行ない、この時の胸管内リンパ液中のNMO濃度を経時的にHexane Bromocresol Purple法により、比色定量した。NMO投与量は10mg per kgとし、これを一度に又は分割して投与した。制癌剤の1回の投与が5～10秒以内に終了する様な投与法をInjection、1時間以上も継続する様な場合をInfusionと呼ぶことにする。NMOの全量を5～10秒以内に1回で投与し終る様にした場合はSingle Injection、全量を分割し、短期間にInjection法を反復するのをRepeated Injectionsと名付ける。

又、全量を1～10時間にわたって1回で持続的に注入した場合をSingle Infusion、全量を分割し、何回かにわたってInfusion法で投与した場合をRepeated

Infusionsと名付ける。これら種々の投与法を行なった場合の成績は次の通りであつた。

1) Catheterを逆行性に大腿動脈より挿入し、腹腔動脈分岐部の高さで大動脈内に留置する場合よりも、腹腔動脈基部内に挿入する場合の方がリンパ液中濃度は高くなる。

2) 1回のSingle Injectionと1回のSingle Infusionの場合とでは、投与開始3時間後に共に最高濃度20γ/ccとなり、投与直後の濃度は極めて高いが、濃度の維持時間は短い。この両者の比較では、Infusion法の方が、やや維持時間が長い。

3) 総量を10回に分割し、1時間に1回の割合で10回くり返すRepeated Injections、あるいは10時間に亘るSingle Infusionの場合では、長時間に亘つて、より一層平坦な濃度を維持しているが、リンパ液中に移行する薬剤濃度は明らかに低くなる。

4) 1回量2～3 mg/kgのInjectionを3～4時間毎にくり返すRepeated Injectionsの場合、比較的高濃度が得られ、かつ長時間に亘り、リンパ液内濃度を一定に保つ事が出来る。

5) 生理的食塩水中では、NMOの活性は、4時間で半減する故、投与前3時間以内に新製したものを使用すべきである。この点からみても、3)の投与法はその都度、NMOを新に調製出来るので便利である。

結局、以上の実験成績の範囲内ではNMOを動脈内に投与する場合に、リンパ液内に持続的に高濃度の薬剤を移行させるためには、3～4時間毎に、新に調製した溶液を分割注射する方法が実用的価値があると思われる。